

Red Records

Best Available Copy



1988-1989 Catalog



**New England Biolabs, Inc.** is a profit-sharing cooperative laboratory of experienced scientists dedicated to providing research products of the highest purity to the scientific community. Our facilities include a large-scale fermentation and production laboratory as well as a fully equipped laboratory for basic research and product development. Products are prepared, analyzed and shipped to you under conditions compatible with product stability and sound environmental policy.

Cover Photograph/ John Lemker

All other photography/David Comb



## T4 DNA Ligase #202

20,000 units \$55  
100,000 units \$220

## #202-C

(Highly Concentrated  
2,000,000 units/ml)

20,000 units \$55  
100,000 units \$220

**Description:** Catalyzes the formation of a phosphodiester bond between juxtaposed 5' phosphate and 3' hydroxyl termini in duplex DNA. This enzyme will join both blunt-ended and cohesive-ended restriction fragments of duplex DNA. The enzyme is purified from *E. coli* C600 pC1857 pPLc28lig 8(1). Contains no detectable exonuclease and endonuclease activity.

**Assay Conditions:** 50 mM Tris-HCl (pH 7.8), 10 mM MgCl<sub>2</sub>, 20 mM dithiothreitol, 1 mM ATP, 50 µg/ml bovine serum albumin and DNA (0.1 to 1 µm in 5' termini). Incubations are at 16°.

**Unit Definitions:** One unit is defined as the amount required to give 50% ligation of *Hind* III fragments of lambda DNA in 30 minutes at 16° in 20 µl of the above assay mixture and a 5' DNA termini concentration of 0.12 µM (300 µg/ml). The example shown indicates a concentration of  $4.0 \times 10^5$  units/ml.

**Blunt-end Ligation:** In general about 50 times as much enzyme is required to achieve the same extent of ligation for blunt-ended as cohesive-ended DNA fragments. Although ligation of blunt-ended DNA fragments requires higher levels of T4 DNA ligase, the concentration of ligase offered by New England Biolabs is more than adequate to achieve greater than 95% ligation of blunt-ended DNA fragments in a short period of time (see figures). Furthermore, the product of the ligase reaction can be fully re-cleaved by the restriction endonuclease which generated the blunt-ended fragments, indicating that the termini are left intact.

**Relationship to Other Ligase Units:** One cohesive end ligation unit (defined above) equals:

- 0.015 ATP-PP exchange unit (2)
- 0.0025 d(A-T) circle formation unit (3)

**Transformation Efficiency After Ligation:** In order to evaluate the T4 DNA ligase under li-

gation conditions used in cloning experiments, the following procedure is performed with the ligase: *E. coli* strain JM101 is transformed with the M13mp8 listed below. Where indicated, the M13mp8 RF DNA is cleaved with *Eco*R I and ligated at the concentration of 4 µg DNA per ml of reaction.

M13mp8 DNA	% Transformants	Ratio colorless/blue plaques
Supercoiled	100%	< 0.1%
<i>Eco</i> R I cleaved	0.5%	< 1.0%
<i>Eco</i> R I cleaved and ligated	20-40%	< 0.1%

The *Eco*R I site of M13mp8 is within the gene encoding beta-galactosidase. Any damage which occurs at the site of ligation and results in a transformant is easily detected on color indicator plates for beta-galactosidase activity. The ratio of colorless/blue plaques indicates the relative number of transformants due to damage at the cleavage site.

**Enzyme Purity:** T4 DNA ligase is run in an SDS polyacrylamide gel system. Each preparation of enzyme is at least 99% pure as indicated by relative band intensities.

**Concentration and Shipping:** 100,000 to 500,000 units/ml. Supplied in 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml bovine serum albumin and 50% glycerol. Store at -20°.

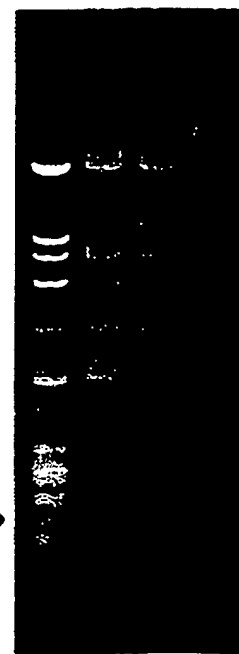
**Reference:** (1) Remaut, E. and Fiers, W., unpublished observations  
(2) Weiss, B., Jacquemin-Sablon, A., Live, T.R., Fareed, G.C. and Richardson, C.C. (1968) *J. Biol. Chem.* 243, 4543-4555  
(3) Modrich, P. and Lehman, I.R. (1970) *J. Biol. Chem.* 245, 3626-3631



0' 10' 20' 30' 60'

Ligation of *Hind* III fragments of Lambda DNA using 1 µl T4 DNA Ligase at a 1:400 dilution

Ligation of *Hae* III fragments of Lambda DNA using various amounts of T4 DNA ligase in a 20 µl reaction volume incubated for 30 minutes at 16°.



0 0.1 0.2 1.0 µl  
T4 DNA Ligase



## T4 RNA Ligase #204

1,000 units	\$44
5,000 units	\$176



**Description:** Catalyzes the ATP-dependent ligation of a 5' phosphoryl-terminated nucleic acid donor to a 3' hydroxyl-terminated nucleic acid acceptor. Substrates include single-stranded RNA and DNA as well as dinucleoside pyrophosphates (1). By optimizing reaction conditions, ligation of single-stranded oligodeoxyribonucleotides of up to 40 bases in length are possible (2). Purified to over 90% homogeneity from *E. coli* RRI containing the plasmid pRF-E35 that overproduces T4 RNA Ligase [constructed at New England Biolabs, Inc. after the method of K.N. Rand and M.J. Gait (3,4)]. Free of detectable levels of single-stranded DNA exonuclease, endonuclease, ribonuclease, and phosphatase.

**Assay Conditions:** 50 mM Tris-HCl (pH 8.0), 10 mM MgCl<sub>2</sub>, 10 µg/ml bovine serum albumin, 20 mM dithiothreitol, 1 mM ATP, 10 µM (5'-<sup>32</sup>P)rA<sub>20</sub> (10 µM in 5' termini) and 0.1 to 0.6 units of enzyme. After incubation at 37°, the reaction is terminated by boiling for 2 minutes, and the bacterial alkaline phosphatase-resistant 5'-phosphoryl termini are determined as described (5).

**Unit Definition:** One unit is defined as the amount required to convert 1 nmole of 5'-phosphoryl termini in (5'-<sup>32</sup>P)rA<sub>20</sub> to a phosphatase-resistant form in 30 minutes at 37° (1) at a 5'-termini concentration of 10 µM (6).

**Concentration and Shipping:** 2,000 to 10,000 units/ml. Supplied in 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml bovine serum albumin and 50% glycerol. Store at -20°.

**Reference:** (1) England, T., Gumpert, R. and Uhlenbeck, O. (1977) *Proc. Nat. Acad. Sci. U.S.A.* 74, 4839-4842  
(2) Tessier, D.C., Brousseau, R. and Vernet, T. (1986) *Anal. Biochem.* 158, 171-178  
(3) Rand, K.N., and Gait, M.J. (1984) *EMBO Journal* vol. 3 no. 2, 397-402  
(4) Strain constructed by Feher, R.  
(5) Sugino, A., Snopek, T.J. and Cozzarelli, N.R. (1977) *J. Biol. Chem.* 252, 1732-1738  
(6) Sugino, A., Goodman, H.M., Heynecker, H.L., Shine, J., Boyer, H.W. and Cozzarelli, N.R. (1977) *J. Biol. Chem.* 252, 3987-3994

## DNA Ligase (*E. coli*, NAD) #205

200 units	\$44
1,000 units	\$176

**Description:** Catalyzes the formation of a phosphodiester bond between juxtaposed 5' phosphate and 3' hydroxyl termini in duplex DNA containing cohesive ends. Required NAD (Nicotinamide adenine dinucleotide) for activity. Purified from *E. coli* strain 594 (su<sup>-</sup>) carrying the prophage λgt4-lop-11 lig<sup>+</sup> Sam 7 (1) by the procedure of Panasenکو et al. (2).

**Assay Conditions:** 30 mM Tris-HCl (pH 8.0), 4 mM MgCl<sub>2</sub>, 0.1 mM EDTA, 20 mM dithiothreitol, 26 µM NAD, 50 µg/ml bovine serum albumin and *Hind* III fragments of lambda DNA (300 to 600 µg/ml). Incubations are at 16°.

**Unit Definition:** One unit is defined as the amount required to give 50% ligation of *Hind* III fragments of lambda DNA in 30 minutes at 16° in 20 µl of the above assay mixture and a 5' DNA termini concentration of 0.12 µM (300 µg/ml).

**Concentration and Shipping:** 1,000 to 4,000 units/ml. Supplied in 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml bovine serum albumin and 50% glycerol. Stable for many months when stored at -20°.

**Reference:** (1) Panasenکو, S.M., Cameron, J.R., Davis, R.W. and Lehman, I.R. (1977) *Science* 196, 188-189  
(2) Panasenکو, S.M., Alazard, R.J. and Lehman, I.R. (1978) *J. Biol. Chem.* 253, 4590-4592